Exploring the Possibilities for Microchip SPE-CE-MS

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Online Peptide Fractionation

Can we modify the ZipChip-SPE-CE-MS method to elute the peptides off of the SPE bed in multiple fractions?

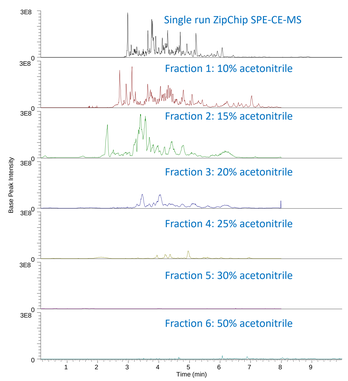
Particles = 5 µm porous silica, C18
BGE = Acetonitrile/Water/Formic acid (ZipChip Peptides BGE)
Weak mobile phase (WMP) = 1% acetonitrile, 0.1 M ammonium acetate

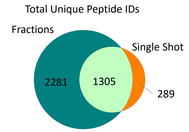
Sample:
- 20 ng/µL HeLa Tryptic digest

Method:
- ZipChip SPE sample loaded as normal (240 seconds)
- Run manually stopped after the Wash step
- Sequence of Standard ZipChip runs performed with increasing % acetonitrile elution solvents in place of samples

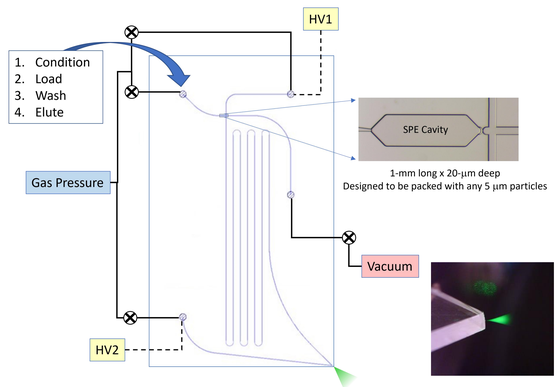
Highlights:
- Successfully eluted different peptides in different fractions
- Total IDs from the fraction runs were ~2x more than running a single ZipChip-SPE run of the same sample

How to improve:
- Adjust acetonitrile content of fractions to more evenly distribute the peptides
- Include a WMP wash before running each fraction
  - This will yield sharper peaks for fractions 2+





Integrated SPE-CE-MS is working well using C18 particles for bottom-up proteomics. What else can we do with this new platform?



Top Down Proteomics

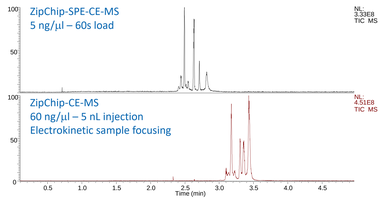
If we swap the C18 particles for more protein-friendly particles, can we use the ZipChip-SPE-CE-MS method for analysis of intact proteins?

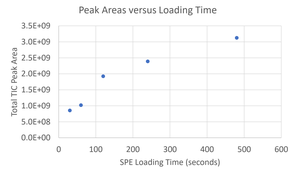
Particles = 5 µm porous polymeric particles
BGE = Acetonitrile/Water/Formic acid (ZipChip Peptides BGE)
WMP = 1% acetonitrile, 0.1 M ammonium acetate

Method:
- Standard ZipChip SPE-CE-MS method

Highlights:
- The proteins in the Pierce Intact Protein mix behaved well with this method
- Concentration factor >100x compared to regular ZipChip without tITP focusing
- Concentration factor >20x compared to regular ZipChip with tITP focusing

How to improve:
- Automate a more thorough rinse of the bed between runs to minimize carryover
- Adjust the solvent compositions to optimize performance for intact proteins





Size Exclusion Desalting for Charge Variant Analysis of mAbs

Instead of solid phase extraction, could we pack the bed with SEC particles and use it for online desalting of Biotherapeutic Proteins

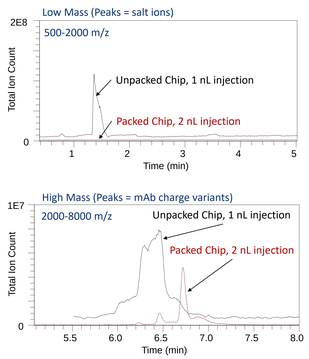
Particles = 5 µm porous silica, Diol
BGE = Acetonitrile/Water/Formic acid (ZipChip Peptides BGE)

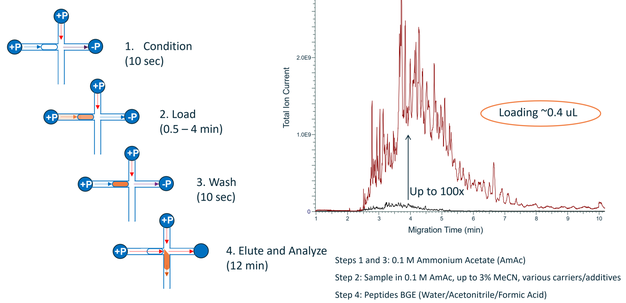
Sample:
- NIST mAb diluted from stock formulation to 0.25 mg/mL with 1x PBS

Method:
- Standard ZipChip method with injection volume setting increased to compensate for resistance of packed bed
- Pressure assist on to back flush salt out of SPE bed during each run

Highlights:
- Effective online desalting of mAb
- Well resolved charge variant peaks from a salty sample

How to improve:
- Use better particles
  - Tighter size distribution and smaller pores
- Make some adjustments to the ZipChip method to achieve more reproducible performance of this application





All data presented here were acquired using a prototype ZipChip Interface (908 Devices) attached to an Exploris 240 mass spectrometer (Thermo). The prototype ZipChip Interface is not commercially available.

Online SPE Desalting of mAbs for Bioreactor Monitoring

Could we use a version of the Top-down method for simple desalting of mAbs pulled directly from a bioreactor?

Particles = 5 µm porous polymeric particles
BGE = Acetonitrile/Water/Formic acid (ZipChip Peptides BGE)
WMP = 1% acetonitrile, 0.1 M ammonium acetate

Samples:
- NIST mAb diluted from stock formulation with CDCHO
- Each sample was further diluted 10x with ZipChip SPE Diluent
- Concentrations listed were before 10x dilution with diluent

Method:
- Standard ZipChip SPE-CE-MS method

Highlights:
- Effective desalting and concentration of mAb
- Clean spectra with accurate deconvolution at 10 µg/mL in CDCHO

How to improve:
- Automate a more thorough rinse of the bed between runs
- Adjust the solvent compositions to optimize performance for intact mAbs
- Explore a combined method that measures unretained small molecules injected through the bed on the same device